TREATMENT OF T-CELL MEDIATED IMMUNE DISORDERS

FIELD

The present disclosure provides methods and compositions for suppressing T cell activation *ex vivo* or *in vivo*. The methods and compositions are useful in the treatment of disorders caused by excessive or aberrant T cell activation, such as autoimmune diseases.

BACKGROUND

The $CD4^+$ T-lymphocyte (herein referred to as the $CD4^+$ T-cell) is the central player in the immune system because of the "help" it provides to other leukocytes in fighting off infection and potential cancerous cells. $CD4^+$ T-cells play essential roles in both humoral and cell-mediated immunity and additionally they act during parasite infection to promote the differentiation of eosinophils and mast cells. If the $CD4^+$ T-cell population is deleted (as is the case in AIDS patients) the host is rendered susceptible to a number of pathogens and tumors that do not ordinarily pose a threat to the host.

While $CD4^+$ T-cells thus play an important beneficial role in disease prevention, the aberrant function of these cells can produce serious problems. In some individuals, the aberrant function of $CD4^+$ T-cells leads to autoimmunity and other disease states. Autoimmune diseases in which $CD4^+$ T-cells have been implicated include multiple sclerosis, rheumatoid arthritis and autoimmune uveitis. In essence these diseases involve an aberrant immune response in which the immune system is subverted from its normal role of attacking invading pathogens and instead attacks the host body tissues, leading to illness and even death. The targeted host tissues vary between autoimmune diseases, for example, in multiple sclerosis the immune system attacks the white matter of the brain and spinal cord, in rheumatoid arthritis the immune system attacks the synovial lining of the joints. Activated $CD4^+$ T-cells have also been implicated in other illnesses, including rejection of transplant tissues and organs and in the development of $CD4^+$ T-cell lymphomas.

Investigations into conditions caused by aberrant $CD4^+$ T-cells activity are focussed on several animal models, and in particular on a number of experimentally induced autoimmune diseases. Research on these experimentally induced diseases in animals is

premised on the idea that they will provide information useful in the treatment of the corresponding human diseases. In pursuit of this goal, it has been shown that $CD4^+$ T-cells are responsible for several experimentally induced autoimmune diseases in animals, including experimental autoimmune encephalomyelitis (EAE), collagen induced arthritis (CIA), and experimental autoimmune uveitis (EAU).

EAE is induced by autoimmunizing animals against myelin basic protein (MBP, a component of the white matter of the brain and the spinal cord) and produces the same clinical symptoms observed in multiple sclerosis: demyelination and paralysis. Proof of the value of the EAE model as a comparative model for multiple sclerosis has been provided by evidence showing that these conditions share a causative nexus: Steinman and co-workers showed that the predominant cell type found in the brain lesions of multiple sclerosis patients is $CD4^+$ T-cells (Oksenberg *et al.*, 1990, Nature 345:344-345) and that the T-cell receptor (the molecule responsible for antigen recognition) associated with the cells in these brain lesions had the same 3 amino acid binding motif for antigen recognition as on the $CD4^+$ T-cells responsible for causing experimental autoimmune encephalomyelitis (EAE) (Oksenberg *et al.*, 1993, Nature 362:68-70). The evidence thus suggests that the EAE model will be useful in testing therapies for disorders caused by aberrant $CD4^+$ T-cell activity.

While it appears that therapeutic approaches that destroy the $CD4^+$ T-cell population might be effective in ameliorating these autoimmune diseases, this approach has one very major drawback. The treatment not only destroys those $CD4^+$ T-cells that are antigen reactive and thus involved in the autoimmune disease process, but also the $CD4^+$ T-cells that are quiescent and not involved in the disease. Since $CD4^+$ T-cells are important in the general immune response (protecting the body against infectious agents), destruction of the entire $CD4^+$ T-cells population leaves the patient severely immunocompromised and hence highly susceptible to infection. A preferable approach would be to suppress activation of $CD4^+$ T-cells in cases of excessive or aberrant $CD4^+$ T-cell activity.

SUMMARY

The present disclosure provides a method for suppressing T cell activation which comprises contacting a cell population comprising T cells *in vitro* or *ex vivo* with an

effective amount of STRO-1^+ cells and/or soluble factors derived therefrom to suppress T cell activation.

In one example the STRO-1^+ cells and/or soluble factors derived therefrom suppress T cell receptor activation.

In one example the cell population is a peripheral blood mononuclear cell sample.

In another example the cell population comprise $CD25^+$ $CD4^+$ T cells of a naïve phenotype ($CD45RA^+$).

In another example the method comprises contacting the cell population comprising T cells *in vitro* or *ex vivo* with an effective amount of STRO-1^+ cells and/or soluble factors derived therefrom and one or more factors which induce formation of regulatory T cells.

The one or more factors which induce formation of regulatory T cells may be selected from the group consisting of α-melanocyte-stimulating hormone (α-MSH), transforming growth factor-β2 (TGF-β2), vitamin D3 and/or Dexamethasone.

In another example the method further comprises contacting the cell population comprising T cells with one or more agents selected from the group consisting of interleukins, antigens, antigen presenting cells, lectins, and antibodies or specific ligands for a cell surface receptors or combinations thereof.

The interleukin may be selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18 or combinations thereof.

The present disclosure also provides a composition of T cells obtained by a method described herein.

The present disclosure also provides a composition comprising T cells, STRO-1^+ cells and/or soluble factors derived therefrom, one or more factors which induce formation of regulatory T cells and a pharmaceutically acceptable carrier.

The present disclosure also provides a method for treating an autoimmune disorder in a subject in need thereof comprising treating a cell population comprising T cells *in vitro* or *ex vivo* with an effective amount of STRO-1^+ cells and/or soluble factors derived therefrom to suppress T cell activation and administering the treated cells to the subject.

The present disclosure also provides a method for treating or preventing a disorder caused by excessive or aberrant T cell activation comprising administering to a subject in need thereof an amount of STRO-1^+ cells and/or soluble factors derived therefrom effective to suppress T-cell activation in the patient.

The method may further comprise administering to the subject one or more agents selected from the group consisting of interleukins, antigens, antigen presenting cells, lectins, and antibodies or specific ligands for a cell surface receptors or combinations thereof. The interleukin may be selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18 or combinations thereof.

Additional active agents that may be conjointly administered with treated T cells or STRO-1^+ cells and/or soluble factors derived therefrom include, but are not limited to, beta-interferons, corticosteroids, non-steroid anti-inflammatory drugs, tumor necrosis alpha blockers, antimalarial drugs, cyclosporines, tumor necrosis alpha inhibitors, immunosuppressants, immunomodulators, antibody therapeutics, cell-based therapies and T cell epitopes (e.g., ToleroTrans Transplant Rejection Therapy by Circassia, etc.).

In some examples the Stro-1^+ cells and/or progeny cells thereof and/or soluble factors derived therefrom are genetically engineered to express a molecule to block co-stimulation of T-cells.

In some examples of the methods of the disclosure the STRO-1^+ cells are enriched for STRO-1^{bright} cells.

The STRO-1^+ cells may be autogeneic or allogeneic. In one example, the STRO-1^{bright} cells are allogeneic.

In another example of this method, the STRO-1^+ cells and/or progeny cells thereof have been expanded in culture prior to obtaining the soluble factors.

In another example of this method the STRO-1^+ cells and/or progeny cells thereof are administered in a dosage ranging from 10^5 to 10^{10} cells.

Exemplary dosages of the cells include between 0.1 x 10^6 to 5 x 10^6 STRO-1^+ cells and/or progeny thereof. For example, the method comprises administering between 0.3 x 10^6 to 2 x 10^6 STRO-1^+ cells and/or progeny thereof.

One form of the method involves administering a low dose of STRO-1^+ cells and/or progeny thereof. Such a low dose is, for example, between 0.1 x 10^5 and 0.5 x 10^6 STRO-1^+ cells and/or progeny thereof, such as about 0.3 x 10^6 STRO-1^+ cells and/or progeny thereof.

The present disclosure also contemplates numerous administrations of the cells and/or soluble factors. For example, such a method can involve administering the cells and monitoring the subject to determine when one or more symptoms of an autoimmune disorder occurs or recurs and administering a further dose of the cells and/or soluble factors. Suitable methods for assessing symptoms of autoimmune disorders will be apparent to the skilled artisan and/or described herein.

In one example, the population enriched for STRO-1^+ cells and/or progeny thereof and/or soluble factors derived therefrom are administered once weekly or less often, such as, once every four weeks or less often.

In another embodiment, the population of cells enriched for STRO-1^{bright} cells and/or progeny cells thereof and/or soluble factors derived therefrom is administered systemically. For example, the population of cells enriched for STRO-1^{bright} cells and/or progeny cells thereof and/or soluble factors derived therefrom may be administered intravenously, intra-arterially, intramuscularly, subcutaneously, into an aorta, into an atrium or ventricle of the heart or into a blood vessel connected to an organ, e.g., an abdominal aorta, a superior mesenteric artery, a pancreaticoduodenal artery or a splenic artery.

In another example the methods of the disclosure further comprise administering an immunosuppressive agent. The immunosuppressive agent may be administered for a time sufficient to permit said transplanted cells to be functional. In one example, the immunosuppressive agent is cyclosporine. The cyclosporine may be administered at a dosage of from 5 to 40 mg/kg body wt.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Brief Description of the Drawings

Figure 1. Co-expression of TNAP (STRO-3) and the Mesenchymal Precursor Cell Marker, STRO-1^{bright} by Adult Human BMMNC. Dual-color immunofluorescence and flow cytometry was performed by incubation of STRO-1 MACS-selected BMMNC and indirectly labelled with a goat anti-murine IgM antibody coupled to FITC (x axis), and STRO-3 mAb (murine IgG1) indirectly labelled with a goat anti-murine IgG coupled to PE (y axis). The dot plot histogram represents 5 x 104 events collected as listmode data. The vertical and horizontal lines were set to the reactivity levels of <1.0% mean fluorescence obtained with the isotype-matched control antibodies, 1B5 (IgG) and 1A6.12 (IgM) treated under the same conditions. The results demonstrate that a minor population of STRO-1^{bright} cells co-expressed TNAP (upper right quadrant) while the remaining STRO-1+ cells failed to react with the STRO-3 mAb.

Figure 2. Gene expression profile of STRO-1^{bright} or STRO-1^{dim} progeny of cultured and expanded STRO-1^{bright} MPC. Single cell suspensions of *ex vivo* expanded bone marrow MPC were prepared by trypsin/EDTA treatment. Cells were stained with the STRO-1 antibody which was subsequently revealed by incubation with goat-anti murine IgM-fluorescein isothiocyanate. Total cellular RNA was prepared from purified populations of STRO-1^{dim} or STRO-1^{bright} expressing cells, following fluorescence activated cell sorting (A). Using RNAzolB extraction method, and standard procedures, total RNA was isolated from each subpopulation and used as a template for cDNA synthesis. The expression of various transcripts was assessed by PCR amplification, using a standard protocol as described previously (Gronthos *et al.* J

Cell Sci. 116:1827-1835, 2003). Primers sets used in this study are shown in Table 2. Following amplification, each reaction mixture was analysed by 1.5% agarose gel electrophoresis, and visualised by ethidium bromide staining (B). Relative gene expression for each cell marker was assessed with reference to the expression of the house-keeping gene, GAPDH, using ImageQant software (C).

Figure 3. STRO-1^{bright} progeny of cultured and expanded STRO-1^{+} MPC express high levels of SDF-1, STRO-1^{dim} progeny do not. (A) MACS-isolated preparations of STRO-1^{+} BMMNCs were partitioned into different STRO-1 subsets according to the regions, STRO-1^{bright} and STRO-1$^{dim/dull}$ using FACS. Total RNA was prepared from each STRO-1 subpopulation and used to construct a STRO-1^{bright} subtraction hybridization library (B-C). Replicate nitrocellulose filters, which have been blotted with representative PCR products amplified from bacterial clones transformed with STRO-1^{bright} subtracted cDNA. The filters were then probed with either [^{32}P] deoxycytidine triphosphate (dCTP)–labeled STRO-1^{bright} (B) or STRO-1$^{dim/dull}$ (C) subtracted cDNA. The arrows indicate differential expression of 1 clone containing a cDNA fragment corresponding to human SDF-1. (D) Reverse transcriptase (RT)–PCR analysis demonstrating the relative expression of SDF-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts in total RNA prepared from freshly MACS/FACS-isolated BMMNC STRO-1 populations prior to culture. bp indicates base pair.

Figure 4. Comparative efficiency of STRO-1 negative MSC (preparation A) and STRO-1^{bright} MPC (preparation B) for inhibition of T cell proliferation. PBMC were stimulated with CD3/CD28 coated beads for 4 days in the absence or presence of preparations A or B. T cell proliferation was measured by ^{3}H-Tdr incorporation as counts per minute (cpm).

Figure 5. Comparative efficiency of STRO-1 negative MSC (preparation A) and STRO-1^{bright} MPC (preparation B) for inhibition of T cell proliferation. PBMC were stimulated with CD3/CD28 coated beads for 4 days in the presence of different concentrations of preparations A or B. T cell proliferation in the various cultures were was measured by ^{3}H-Tdr incorporation and reported as percentage of the control T cell proliferation in which PBMC were stimulated in the absence of MSC.

Figure 6. STRO-1^{bright} MPC reduce or prevent T cell immune response to a specific antigen. Splenocytes were obtained from MPC treated mice and controls on day 36 after MOG_{35-55} immunization. Splenocytes were cultured *in vitro* and restimulated with MOG_{35-55} and T-cell proliferative responses were measured through [^{3}H]-thymidine incorporation.

Figure 7. STRO-3 immunoselected and culture expanded human MPCs inhibit PHA activation of T cells. PMBC were stimulated with phytohemagglutinin (PHA) to illicit lymphocyte proliferation. STRO-3 selected MPCs were able to significantly suppress PMBC T-cell proliferation over a range of concentrations.

Figure 8. STRO-3 immunoselected and culture expanded ovine STRO-1^{bright} cells (MPCs) induce very low levels of alloimmune responses, and inhibit PHA activation of T cells. Ovine MPCs did not directly induce lymphcyte proliferation when used at a stimulator cell at 1%, 5%, 10% 20% or 50% dilutions and were able to significantly inhibit levels of alloimmune responses to ovine PBMCs stimulated with PHA.

Figure 9. Dose dependent immunosuppressive effects of ovine STRO-3 immunoselected and culture expanded STRO-1^{bright} cells (MPCs) on PHA-mediated lymphocyte proliferation. Ovine MPCs were able to suppress lymphocyte proliferation in a dose dependent manner when used as stimulators against ovine PBMCs or purified ovine T cells (selected using Miltenyi T cell isolation kit).

DETAILED DESCRIPTION

The present disclosure demonstrates that STRO-1$^+$ cells inhibit T cell activation via antigen and non-antigen specific mechanisms. For example, the present disclosure demonstrates that STRO-1$^+$ cells inhibit MOG- specific T cell proliferative responses *in vivo*. The present disclosure also demonstrates that STRO-1$^+$ cells inhibit anti-CD3 mediated T cell proliferative responses *in vitro*. This disclosure therefore provides new therapeutic approaches for treating or preventing diseases where aberrations in regulatory T cell number and/or function have been observed (e.g., in autoimmune disorders).

Definitions

As used herein, the terms "treatment", "treating", and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disorder or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disorder (e.g., autoimmune disease) and/or adverse affect attributable to the disorder. "Treatment", as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) increasing survival time; (b) decreasing the risk of death due to the disease; (c) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (d) inhibiting the disease, i.e., arresting its development (e.g., reducing the rate of disease progression); and (e) relieving the disease, i.e., causing regression of the disease.

As used herein, a therapeutic that "prevents" a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample.

As used herein the terms "subject" and "patient" refer to animals including mammals, including humans. The term "mammal" includes primates, domesticated animals including dogs, cats, sheep, cattle, horses, goats, pigs, mice, rats, rabbits, guinea pigs, captive animals such as zoo animals, and wild animals.

Treatment Methods

The present disclosure provides methods for suppressing T cell activation that can be effected *in vitro* or *in vivo*.

In some embodiments the method for suppressing T cell activation comprises contacting a cell population comprising T cells *in vitro* or *ex vivo* with an effective amount of STRO-1^+ cells and/or soluble factors derived therefrom for a period of time sufficient to suppress T cell activation.

In some embodiments, the method comprises obtaining a cell population that comprises T cells (e.g., $CD4^+$ cells) and contacting the T cells with STRO-1^+ cells and/or soluble factors derived therefrom for a period of time sufficient to suppress T cell receptor activation.

In one embodiment the STRO-1^+ cells and/or soluble factors derived therefrom stimulate formation or expansion of regulatory T cells within the cell population. Regulatory T cells are a subset of T cells that suppress the activity of effector T cells and are characterized by the markers $CD4^+$ $CD25^+$. In some embodiments, the regulatory T cells are $FoxP3^+$ and/or IL-10 producing regulatory T cells.

Accordingly, in a further embodiment the method of the disclosure comprises culturing the cell population comprising T cells *in vitro* or *ex vivo* with an effective amount of STRO-1^+ cells and/or soluble factors derived therefrom and one or more factors that stimulate formation of regulatory T cells, such as α-melanocyte-stimulating hormone (α-MSH) and/or transforming growth factor-β2 (TGF- β2). In some aspects, the culture also contain vitamin D3 and/or Dexamethasone, which have demonstrated to promote the generation of IL-10-producing regulatory T cells (Barrat et al. J. Exp. Med. 195(5): 2002, 603-616).

In some embodiments, the T cells are isolated from a mammalian sample prior to exposure to STRO-1^+ cells and/or soluble factors derived therefrom.

The term "isolated" with respect to T cells refers to cell population preparation in a form that has at least 70, 80, 90, 95, 99, or 100% T cells. In some aspects, a desired cell population is isolated from other cellular components, in some instances to specifically exclude other cell types that may "contaminate" or interfere with the study of the cells in isolation. It is to be understood, however, that such an "isolated" cell population may incorporate additional cell types that are necessary for cell survival or to achieve the desired results provided by the disclosure. For example, antigen presenting cells, such as monocytes (macrophages) or dendritic cells, may be present in an "isolated" cell population of T cells or added to a population of isolated T cells for generation of regulatory T cells. In some aspects, these antigen presenting cells may be activated monocytes or dendritic cells.

Cell populations comprising T cells for use in the methods of the disclosure may be isolated from a biological sample taken from a mammalian subject. The sample may originate from a number of sources, including, but not limited to peripheral blood, leukapheresis blood product, apheresis blood product, bone marrow, thymus, tissue biopsy, tumor, lymph node tissue, gut associated lymphoid tissue, mucosa associated lymphoid tissue, liver, sites of immunologic lesions (e.g., synovial fluid), pancreas, and cerebrospinal fluid. The donor subject is preferably human, and can be fetal, neonatal, child, adult, and may be normal, diseased, or susceptible to a disease of interest.

In some embodiments, the T cell sample comprises peripheral blood mononuclear cells (PBMCs) from a blood sample. By "peripheral blood mononuclear cells" or "PBMCs" is meant lymphocytes (including T-cells, B-cells, NK cells, etc.) and monocytes. In general, PBMCs are isolated from a patient using standard techniques. In some embodiments, only PBMCs are taken, either leaving or returning substantially all of the red blood cells and polymorphonuclear leukocytes to the donor. PBMCs may be isolated using methods known in the art, such as leukophoresis. In general, a 5 to 7 liter leukophoresis step is performed, which essentially removes PBMCs from a patient, returning the remaining blood components. Collection of the sample is preferably performed in the presence of an anticoagulant (e.g., heparin).

The T cell-containing sample comprising PBMCs or isolated T cells can be pretreated using various methods before treatment with STRO-1^+ cells and/or soluble factors derived therefrom. Generally, once collected, the cells can be additionally concentrated, if this was not done simultaneously with collection or to further purify and/or concentrate the cells. For example, PBMCs can be partially purified by density gradient centrifugation (e.g., through a Ficoll-Hypaque gradient). Cells isolated from a donor sample are normally washed to remove serum proteins and soluble blood components, such as autoantibodies, inhibitors, etc., using techniques well known in the art. Generally, this involves addition of physiological media or buffer, followed by centrifugation. This may be repeated as necessary. The cells can then be counted, and in general, from 1 x 10^9 to 2 x 10^9 white blood cells are collected from a 5-7 liter leukapheresis. The purified cells can be resuspended in suitable media or buffer to maintain viability. Suitable solutions for resuspension will generally be a balanced salt solution (e.g., normal saline, PBS, Hank's balanced salt solution, etc.) optionally supplemented with fetal calf serum, BSA, HSA, normal goat serum, and/or other naturally occurring factors, in conjunction with an acceptable buffer at low

concentration, generally from 5-50 mM. Convenient buffers include, but are not limited to HEPES, phosphate buffers, lactate buffers, etc.

A specific cell type (e.g., effector T cells, regulatory T cells, etc.) can be separated from a complex mixture of cells using techniques that enrich for cells having the desired characteristic (e.g., $CD4^+$, $FoxP3^+$, etc.). Most standard separation methods use affinity purification techniques to obtain a substantially isolated cell population. Techniques for affinity separation may include, but are not limited to, magnetic separation (e.g., using antibody-coated magnetic beads), affinity chromatography, cytotoxic agents joined to a monoclonal antibody (e.g., complement and cytotoxins), and "panning" with antibody attached to a solid matrix. Techniques providing accurate separation include fluorescence activated cell sorting, which can have varying degrees of sophistication, such as multiple color channels, impedance channels, etc. The living cells may be selected against dead cells by employing dyes that associate with dead cells (e.g., propidium iodide, LDS, etc.). Any technique may be used that is not unduly detrimental to the viability of the selected cells.

The affinity reagents used may be specific receptors or ligands for cell surface molecules (e.g., CD4, CD25, etc.). Antibodies may be monoclonal or polyclonal and may be produced by transgenic animals, immunized animals, immortalized B-cells, and cells transfected with DNA vectors encoding the antibody. Details of the preparation of antibodies and their suitability for use as specified binding members are well-known to those skilled in the art. In addition to antibody reagents, peptide-MHC antigen and T cell receptor pairs may be used, as well as peptide ligands, effector and receptor molecules.

Antibodies used as affinity reagents for purification are generally conjugated with a label for use in separation. Labels may include magnetic beads (which allow for direct separation), biotin (which can be removed with avidin or streptavidin bound to a support), fluorochromes (which can be used with a fluorescence activated cell sorter), or other such labels that allow for ease of separation of the particular cell type. Fluorochromes may include phycobiliproteins, such as phycoerythrin and allophycocyanins, fluorescein and Texas red. Frequently, each antibody is labeled with a different fluorochrome to permit independent sorting for each marker.

For purification of a desired cell population, cell-specific antibodies are added to a suspension of cells and incubated for a period of time sufficient to bind the available cell surface antigens. The incubation will usually be at least about 5 minutes and usually less than about 30 minutes. It is desirable to have a sufficient concentration of antibodies in the reaction mixture, such that the efficiency of the separation is not limited by lack of antibody (i.e., using a saturating amount of antibody). The appropriate concentration can also be determined by titration. The medium in which the cells are separated will be any medium that maintains the viability of the cells. A preferred medium is phosphate buffered saline containing from 0.1% to 0.5% BSA. Various media are commercially available and may be used according to the nature of the cells, including Dulbecco's Modified Eagle Medium, Hank's Basic Salt Solution, Dulbecco's phosphate buffered saline, RPMI, Iscove's medium, PBS with 5 mM EDTA, etc., optionally supplemented with fetal calf serum, BSA, HSA, etc.

The staining intensity of cells can be monitored by flow cytometry, where lasers detect the quantitative levels of fluorochrome (which is proportional to the amount of cell surface antigen bound by the antibodies). Flow cytometry, or fluorescent activated cell sorting (FACS), can also be used to separate cell populations based on the intensity of antibody staining, as well as other parameters such as cell size and light scatter. Although the absolute level of staining may differ with a particular fluorochrome and antibody preparation, the data can be normalized to a control.

The labeled cells are then separated as to the expression of designated marker (e.g., CD4, CD25, etc.). The separated cells may be collected in any appropriate medium that maintains the viability of the cells, usually having a cushion of serum at the bottom of the collection tube. Various media are commercially available and may be used according to the nature of the cells, including dMEM, HBSS, dPBS, RPMI, Iscove's medium, etc., frequently supplemented with fetal calf serum.

Cell populations highly enriched for a desired characteristic (e.g., $CD4^+$ T cells, $CD4^+CD25^+$ regulatory T cells, etc.) are achieved in this manner. The desired population will be at or about 70% or more of the cell composition, and usually at or about 90% or more of the cell composition, and may be as much as about 95% or more of the cell population. The enriched cell population may be used immediately. Cells can also be frozen, although it is preferable to freeze cells prior to the separation procedure. Alternatively, cells may be frozen at liquid nitrogen temperatures and stored

for long periods of time, being thawed and capable of being reused. The cells will usually be stored in DMSO and/or FCS, in combination with medium, glucose, etc. Once thawed, the cells may be expanded by use of growth factors, antigen, stimulation, antigen presenting cells (e.g., dendritic cells), etc. for proliferation and differentiation.

Once the PBMCs or isolated T cells have undergone any necessary pre-treatment, the cells are treated with STRO-1^+ cells and/or soluble factors derived therefrom. By "treated" herein is meant that the cells are incubated in a suitable nutrient medium with STRO-1^+ cells and/or soluble factors derived therefrom for a time period sufficient to produce regulatory T cells having the capacity to inhibit immune responses mediated by effector T cells. In some embodiments, the first culture is diluted with about an equal volume of nutrient medium. In other aspects, a first cell culture is divided into two or more portions that are then diluted with nutrient medium. The advantage of culture division is that the cell clusters formed in the first culture (thousands of cells) are mechanically disrupted and form smaller cell clusters (tens to hundreds of cells) during division of the first culture. These small clusters are then able to grow into larger clusters during the next growth period. A cell culture produced in this fashion may be subcultured two or more times using a similar method.

A cell population may be grown *in vitro* under various culture conditions. Culture medium may be liquid or semi-solid (e.g., containing agar, methylcellulose, etc.) The cell population may be conveniently suspended in any appropriate nutrient medium, including but not limited to Iscove's modified Dulbecco's medium, or RPMI-1640, normally supplemented with fetal calf serum (about 5-10%), L-glutamine, and antibiotics (e.g., penicillin and streptomycin).

The cell culture may contain growth factors to which the cells are responsive. Growth factors, as defined herein, are molecules capable of promoting survival, growth and/or differentiation of cells, either in culture or in the intact tissue, through specific effects on a transmembrane receptor. Growth factors include polypeptides and non-polypeptide factors. Specific growth factors that may be used in culturing the subject cells include the interleukins (e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, etc.) and antigens (e.g., peptide antigens, protein antigens such as alloantigens) preferably in combination with antigen presenting cells, lectins, non-specific stimuli (e.g., Con A; LPS; etc.). The culture may also contain antibodies (e.g. anti-CD3), or specific ligands (in the form of

purified ligand, Fc fusion proteins, or other recombinant tagged forms like leucine zipper forms) for cell surface receptors that may stimulate regulatory T cell activity. For example, mAb or ligands that bind TNFR or other co-stimulatory molecules on regulatory T cells and could stimulate and increase regulatory T cell activity.

The T cell population may be co-cultured with immature or mature dendritic cells, as well as other antigen presenting cells (e.g., monocytes, B cells, macrophages, etc.) prior to, during, or after treatment STRO-1^+ cells and/or soluble factors derived therefrom.

In some aspects, the present methods are useful for *ex vivo* generation of regulatory T cells for transplantation into a patient or development of in vitro models and assays for regulatory T cell function. The regulatory T cell cultures serve as a valuable source of novel regulatory factors and pharmaceuticals.

Methods for adoptive transfer of T cells are well described in the art, for example, see US Patent Applications 2006/0115899, 2005/0196386, 2003/0049696, 2006/0292164, and 2007/0172947 (the contents of which are hereby incorporated by reference). Therefore, a skilled practitioner would easily be able to transplant or reintroduce the treated T cells populations obtained by the methods of the present disclosure into a patient in need thereof. Transplanted T cells may originate from a T cell-containing sample obtained from the patient himself or from another donor not receiving treatment. This is generally done as is known in the art and usually comprises injecting, or other methods of introducing, the treated cells back into the patient via intravenous administration. For example, the cells may be placed in a 50 ml Fenwall infusion bag by injection using sterile syringes or other sterile transfer mechanisms. The cells can then be immediately infused via IV administration over a period of time, into a free flow IV line into the patient. In some aspects, additional reagents such as buffers or salts may be added as well.

Another aspect of the disclosure provides methods for treating autoimmune-related disorders by conjoint administration of treated T cells obtained by a method of the present disclosure and at least one additional active agent. In some embodiments, the additional active agent is a therapeutic agent used to treat or prevent an autoimmune disease. Active agents of the invention may include, but are not limited to beta-interferons, corticosteroids, non-steroid anti-inflammatory drugs, tumor necrosis blockers, antimalarial drugs, cyclosporines, tumor necrosis alpha inhibitors,

immunosuppressants, immunomodulators, cytokines, anti-graft-rejection therapeutics, vitamin D3, Dexamethasone, antibody therapeutics, and T cell epitopes (e.g., ToleroTrans Transplant Rejection Therapy by Circassia, etc.). Cytokines suitable for conjoint administration may include, but are not limited to IL-2, IL-4, IL-7, IL-10, TGF-β, IL-15 and/or IL-17. In some embodiments the additional active agent may be a cell population comprising other cell types than regulatory T cells. For example, the treated T cells may be conjointly administered to a patient in need thereof with one or more antigen presenting cell types, such as monocytes or dendritic cells. In some aspects, these antigen presenting cells may be activated monocytes or dendritic cells.

After transplanting the cells into the patient, the effect of the treatment may be evaluated, if desired. One of skill in the art would recognize there are many methods of evaluating immunological manifestations of an autoimmune disease (e.g., quantification of total antibody titers or of specific immunoglobulins, renal function tests, tissue damage evaluation, etc.). Tests of T cells function such as T cell numbers, phenotype, activation state and ability to respond to antigens and/or mitogens also may be done.

One aspect of the disclosure provides methods for treating or preventing a disorder caused by excessive T cell activation, such as an autoimmune disorder or condition, in a patient by administering to a patient in need thereof an amount of STRO-1^+ cells and/or soluble factors derived therefrom effective to suppress T-cell activation in the patient.

The examples of the disclosure demonstrate that administration of STRO-1^+ cells and/or soluble factors derived therefrom to a mouse model resulted in suppression of effector T cell activity.

In one embodiment the present disclosure provides methods of administering STRO-1^+ cells and/or soluble factors derived therefrom to promote regulatory T cell-mediated suppression of autoimmune disorders or conditions.

While the method of the invention can be used to treat patients afflicted with an autoimmune disorder, in some embodiments, the methods are also applied to patients who do not have, but are at risk of developing an autoimmune response.

The present disclosure provides methods for treating autoimmune-related disorders by conjoint administration of STRO-1$^+$ cells and/or soluble factors derived therefrom and at least one additional active agent. Active agents of the invention may include, but are not limited to beta-interferons, corticosteroids, non-steroid anti-inflammatory drugs, tumor necrosis blockers, antimalarial drugs, cyclosporines, tumor necrosis alpha inhibitors, immunosuppressants, immunomodulators, cytokines, anti-graft-rejection therapeutics, cell-based therapeutics, vitamin D3, dexamethasone and antibody therapeutics. Cytokines suitable for conjoint administration may include, but are not limited to IL-2, IL-4, IL-10, TGF-β, IL-15 and/or IL-17. In some embodiments, the additional active agent is a therapeutic agent used to treat or prevent an autoimmune disease.

The pathogenesis of a number of autoimmune diseases is believed to be caused by autoimmune T cell responses to self-antigens present in the organism. For example, autoreactive T cells have been implicated in the pathogenesis of: type I diabetes, multiple sclerosis, rheumatoid arthritis, psoriatic arthritis, autoimmune myocarditis, pemphigus, celiac disease, myasthenia gravis, Hashimoto's thyroiditis, Graves' disease, Addison's disease, autoimmune hepatitis, chronic Lyme arthritis, familial dilated cardiomyopathy, juvenile dermatomyositis, polychondritis, Sjogren's syndrome, psoriasis, juvenile idiopathic arthritis, inflammatory bowel disease, systemic lupus erythematosus, and graft-versus-host disease.

As used herein, the term “soluble factors” shall be taken to mean any molecule, e.g., protein, peptide, glycoprotein, glycopeptide, lipoprotein, lipopeptide, carbohydrate, etc. produced by STRO-1$^+$ cells and/or progeny thereof that are water soluble. Such soluble factors may be intracellular and/or secreted by a cell. Such soluble factors may be a complex mixture (e.g., supernatant) and/or a fraction thereof and/or may be a purified factor. In one embodiment of the present invention soluble factors are or are contained within supernatant. Accordingly, any embodiment herein directed to administration of one or more soluble factors shall be taken to apply mutatis mutandis to the administration of supernatant.

The methods of the invention may involve administration of population of cells enriched for STRO-1$^+$ cells and/or progeny cells thereof alone, and/or soluble factors derived therefrom. The methods of the invention may also involve administration of progeny cells alone, or soluble factors derived from the progeny cells. The methods of

the invention may also involve administration of a mixed population of STRO-1^{bri} cells and progeny cells thereof, or soluble factors from a mixed culture of STRO-1^{bri} cells and progeny cells thereof.

It is further contemplated that only a single treatment with the STRO-1^+ cells and/or progeny cells thereof and/or soluble factors derived therefrom of the present invention may be required, eliminating the need for chronic immunosuppressive drug therapy. Alternatively, multiple administrations of STRO-1^+ cells and/or progeny cells thereof and/or soluble factors derived therefrom may be employed.

The dosage of the STRO-1^+ cells and/or progeny cells thereof and/or soluble factors derived therefrom varies within wide limits and will, of course be fitted to the individual requirements in each particular case. In general, in the case of parenteral administration, it is customary to administer from about 0.01 to about 5 million cells per kilogram of recipient body weight. The number of cells used will depend on the weight and condition of the recipient, the number of or frequency of administrations, and other variables known to those of skill in the art

Exemplary dosages of the cells include between 0.1 x 10^6 to 5 x 10^6 STRO-1^+ cells and/or progeny thereof. For example, the method comprises administering between 0.3 x 10^6 to 2 x 10^6 STRO-1^+ cells and/or progeny thereof.

One form of the method involves administering a low dose of STRO-1^+ cells and/or progeny thereof. Such a low dose is, for example, between 0.1 x 10^5 and 0.5 x 10^6 STRO-1^+ cells and/or progeny thereof, such as about 0.3 x 10^6 STRO-1^+ cells and/or progeny thereof.

The cells can be suspended in an appropriate diluent, at a concentration of from about 0.01 to about 5 x 10^6 cells/ml. Suitable excipients for injection solutions are those that are biologically and physiologically compatible with the cells and with the recipient, such as buffered saline solution or other suitable excipients. The composition for administration is preferably formulated, produced and stored according to standard methods complying with proper sterility and stability.

The following examples further illustrate aspects of the present invention. However, they are in no way a limitation of the teachings or disclosure of the present invention as set forth herein.

STRO-1$^+$ Cells or Progeny Cells, and Supernatant or One or More Soluble Factors Derived Therefrom

STRO-1$^+$ cells are cells found in bone marrow, blood, dental pulp cells, adipose tissue, skin, spleen, pancreas, brain, kidney, liver, heart, retina, brain, hair follicles, intestine, lung, lymph node, thymus, bone, ligament, tendon, skeletal muscle, dermis, and periosteum; and are capable of differentiating into germ lines such as mesoderm and/or endoderm and/or ectoderm.

In one embodiment, the STRO-1$^+$ cells are multipotential cells which are capable of differentiating into a large number of cell types including, but not limited to, adipose, osseous, cartilaginous, elastic, muscular, and fibrous connective tissues. The specific lineage-commitment and differentiation pathway which these cells enter depends upon various influences from mechanical influences and/or endogenous bioactive factors, such as growth factors, cytokines, and/or local microenvironmental conditions established by host tissues. STRO-1$^+$ multipotential cells are thus non-hematopoietic progenitor cells which divide to yield daughter cells that are either stem cells or are precursor cells which in time will irreversibly differentiate to yield a phenotypic cell.

In one example, the STRO-1$^+$ cells are enriched from a sample obtained from a subject, e.g., a subject to be treated or a related subject or an unrelated subject (whether of the same species or different). The terms 'enriched', 'enrichment' or variations thereof are used herein to describe a population of cells in which the proportion of one particular cell type or the proportion of a number of particular cell types is increased when compared with an untreated population of the cells (e.g., cells in their native environment).

In one example, the cells used in the present disclosure express one or more markers individually or collectively selected from the group consisting of TNAP$^+$, VCAM-1$^+$, THY-1$^+$, STRO-4$^+$ (HSP-90β), STRO-2$^+$, CD45$^+$, CD146$^+$, 3G5$^+$ or any combination thereof.

By "individually" is meant that the disclosure encompasses the recited markers or groups of markers separately, and that, notwithstanding that individual markers or groups of markers may not be separately listed herein the accompanying claims may define such marker or groups of markers separately and divisibly from each other.

By "collectively" is meant that the disclosure encompasses any number or combination of the recited markers or groups of peptides, and that, notwithstanding that such numbers or combinations of markers or groups of markers may not be specifically listed herein the accompanying claims may define such combinations or sub-combinations separately and divisibly from any other combination of markers or groups of markers.

In one example, the STRO-1^{+} cells are STRO-1^{bright} (*syn*. STRO-1^{bri}). In one example, the Stro-1^{bri} cells are preferentially enriched relative to STRO-1^{dim} or STRO-$1^{intermediate}$ cells.

In one example, the STRO-1^{bright} cells are additionally one or more of $TNAP^{+}$, VCAM-1^{+}, THY-1^{+}, STRO-4^{+} (HSP-90β), STRO-2^{+} and/or $CD146^{+}$.

In one example, the mesenchymal precursor cells are perivascular mesenchymal precursor cells as defined in WO 2004/85630.

A cell that is referred to as being "positive" for a given marker it may express either a low (lo or dim) or a high (bright, bri) level of that marker depending on the degree to which the marker is present on the cell surface, where the terms relate to intensity of fluorescence or other marker used in the sorting process of the cells. The distinction of lo (or dim or dull) and bri will be understood in the context of the marker used on a particular cell population being sorted. A cell that is referred to as being "negative" for a given marker is not necessarily completely absent from that cell. This term means that the marker is expressed at a relatively very low level by that cell, and that it generates a very low signal when detectably labeled or is undetectable above background levels, e.g., levels detected suing an isotype control antibody.

The term "bright", when used herein, refers to a marker on a cell surface that generates a relatively high signal when detectably labeled. Whilst not wishing to be limited by theory, it is proposed that "bright" cells express more of the target marker protein (for

example the antigen recognized by STRO-1) than other cells in the sample. For instance, STRO-1^{bri} cells produce a greater fluorescent signal, when labeled with a FITC-conjugated STRO-1 antibody as determined by fluorescence activated cell sorting (FACS) analysis, than non-bright cells (STRO-$1^{dull/dim}$). In one example, "bright" cells constitute at least about 0.1% of the most brightly labeled bone marrow mononuclear cells contained in the starting sample. In other examples, "bright" cells constitute at least about 0.1%, at least about 0.5%, at least about 1%, at least about 1.5%, or at least about 2%, of the most brightly labeled bone marrow mononuclear cells contained in the starting sample. In an example, STRO-1^{bright} cells have 2 log magnitude higher expression of STRO-1 surface expression relative to "background", namely cells that are STRO-1^{-}. By comparison, STRO-1^{dim} and/or STRO-$1^{intermediate}$ cells have less than 2 log magnitude higher expression of STRO-1 surface expression, typically about 1 log or less than "background".

As used herein the term "TNAP" is intended to encompass all isoforms of tissue non-specific alkaline phosphatase. For example, the term encompasses the liver isoform (LAP), the bone isoform (BAP) and the kidney isoform (KAP). In an example, the TNAP is BAP. In an example, TNAP as used herein refers to a molecule which can bind the STRO-3 antibody produced by the hybridoma cell line deposited with ATCC on 19 December 2005 under the provisions of the Budapest Treaty under deposit accession number PTA-7282.

Furthermore, in an example, the STRO-1^{+} cells are capable of giving rise to clonogenic CFU-F.

In one example, a significant proportion of the STRO-1^{+} multipotential cells are capable of differentiation into at least two different germ lines. Non-limiting examples of the lineages to which the multipotential cells may be committed include bone precursor cells; hepatocyte progenitors, which are multipotent for bile duct epithelial cells and hepatocytes; neural restricted cells, which can generate glial cell precursors that progress to oligodendrocytes and astrocytes; neuronal precursors that progress to neurons; precursors for cardiac muscle and cardiomyocytes, glucose-responsive insulin secreting pancreatic beta cell lines. Other lineages include, but are not limited to, odontoblasts, dentin-producing cells and chondrocytes, and precursor cells of the following: retinal pigment epithelial cells, fibroblasts, skin cells such as keratinocytes, dendritic cells, hair follicle cells, renal duct epithelial cells, smooth and skeletal muscle

cells, testicular progenitors, vascular endothelial cells, tendon, ligament, cartilage, adipocyte, fibroblast, marrow stroma, cardiac muscle, smooth muscle, skeletal muscle, pericyte, vascular, epithelial, glial, neuronal, astrocyte and oligodendrocyte cells.

In another example, the STRO-1$^+$ cells are not capable of giving rise, upon culturing, to hematopoietic cells.

In one example, the cells are taken from the subject to be treated, cultured *in vitro* using standard techniques and used to obtain supernatant or soluble factors or expanded cells for administration to the subject as an autologous or allogeneic composition. In an alternative example, cells of one or more of the established human cell lines are used. In another useful example of the disclosure, cells of a non-human animal (or if the patient is not a human, from another species) are used.

The present disclosure also contemplates use of supernatant or soluble factors obtained or derived from STRO-1$^+$ cells and/or progeny cells thereof (the latter also being referred to as expanded cells) which are produced from *in vitro* culture. Expanded cells of the disclosure may a have a wide variety of phenotypes depending on the culture conditions (including the number and/or type of stimulatory factors in the culture medium), the number of passages and the like. In certain examples, the progeny cells are obtained after about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 passages from the parental population. However, the progeny cells may be obtained after any number of passages from the parental population.

The progeny cells may be obtained by culturing in any suitable medium. The term "medium", as used in reference to a cell culture, includes the components of the environment surrounding the cells. Media may be solid, liquid, gaseous or a mixture of phases and materials. Media include liquid growth media as well as liquid media that do not sustain cell growth. Media also include gelatinous media such as agar, agarose, gelatin and collagen matrices. Exemplary gaseous media include the gaseous phase that cells growing on a petri dish or other solid or semisolid support are exposed to. The term "medium" also refers to material that is intended for use in a cell culture, even if it has not yet been contacted with cells. In other words, a nutrient rich liquid prepared for bacterial culture is a medium. A powder mixture that when mixed with water or other liquid becomes suitable for cell culture may be termed a "powdered medium".

In an example, progeny cells useful for the methods of the disclosure are obtained by isolating $TNAP^+$ $STRO\text{-}1^+$ cells from bone marrow using magnetic beads labeled with the STRO-3 antibody, and then culture expanding the isolated cells (see Gronthos *et al. Blood 85*: 929-940, 1995 for an example of suitable culturing conditions).

In one example, such expanded cells (progeny) (for example, at least after 5 passages) can be $TNAP^-$, $CC9^+$, HLA class I^+, HLA class II^-, $CD14^-$, $CD19^-$, $CD3^-$, $CD11a^-c^-$, $CD31^-$, $CD86^-$, $CD34^-$ and/or $CD80^-$. However, it is possible that under different culturing conditions to those described herein that the expression of different markers may vary. Also, whilst cells of these phenotypes may predominate in the expended cell population it does not mean that there is a minor proportion of the cells do not have this phenotype(s) (for example, a small percentage of the expanded cells may be $CC9^-$). In one example, expanded cells still have the capacity to differentiate into different cell types.

In one example, an expended cell population used to obtain supernatant or soluble factors, or cells *per se*, comprises cells wherein at least 25%, such as at least 50%, of the cells are $CC9^+$.

In another example, an expanded cell population used to obtain supernatant or soluble factors, or cells *per se*, comprises cells wherein at least 40%, such as at least 45%, of the cells are $STRO\text{-}1^+$.

In a further example, the expanded cells may express one or more markers collectively or individually selected from the group consisting of LFA-3, THY-1, VCAM-1, ICAM-1, PECAM-1, P-selectin, L-selectin, 3G5, CD49a/CD49b/CD29, CD49c/CD29, CD49d/CD29, CD 90, CD29, CD18, CD61, integrin beta 6-19, thrombomodulin, CD10, CD13, SCF, PDGF-R, EGF-R, IGF1-R, NGF-R, FGF-R, Leptin-R (STRO-2 = Leptin-R), RANKL, $STRO\text{-}1^{bright}$ and CD146 or any combination of these markers.

In one example, the progeny cells are Multipotential Expanded $STRO\text{-}1^+$ Multipotential cells Progeny (MEMPs) as defined and/or described in WO 2006/032092. Methods for preparing enriched populations of $STRO\text{-}1^+$ multipotential cells from which progeny may be derived are described in WO 01/04268 and WO 2004/085630. In an *in vitro* context $STRO\text{-}1^+$ multipotential cells will rarely be present as an absolutely pure preparation and will generally be present with other cells that are tissue specific

committed cells (TSCCs). WO 01/04268 refers to harvesting such cells from bone marrow at purity levels of about 0.1% to 90%. The population comprising MPCs from which progeny are derived may be directly harvested from a tissue source, or alternatively it may be a population that has already been expanded *ex vivo*.

For example, the progeny may be obtained from a harvested, unexpanded, population of substantially purified STRO-1$^+$ multipotential cells, comprising at least about 0.1, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80 or 95% of total cells of the population in which they are present. This level may be achieved, for example, by selecting for cells that are positive for at least one marker individually or collectively selected from the group consisting of TNAP, STRO-1^{bright}, 3G5$^+$, VCAM-1, THY-1, CD146 and STRO-2.

MEMPS can be distinguished from freshly harvested STRO-1$^+$ multipotential cells in that they are positive for the marker STRO-1^{bri} and negative for the marker Alkaline phosphatase (ALP). In contrast, freshly isolated STRO-1$^+$ multipotential cells are positive for both STRO-1^{bri} and ALP. In an example of the present disclosure, at least 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of the administered cells have the phenotype STRO-1^{bri}, ALP$^-$. In one example the MEMPS are positive for one or more of the markers Ki67, CD44 and/or CD49c/CD29, VLA-3, $\alpha 3\beta 1$. In yet a further example the MEMPs do not exhibit TERT activity and/or are negative for the marker CD18.

The STRO-1$^+$ cell starting population may be derived from any one or more tissue types set out in WO 01/04268 or WO 2004/085630, namely bone marrow, dental pulp cells, adipose tissue and skin, or perhaps more broadly from adipose tissue, teeth, dental pulp, skin, liver, kidney, heart, retina, brain, hair follicles, intestine, lung, spleen, lymph node, thymus, pancreas, bone, ligament, bone marrow, tendon and skeletal muscle.

It will be understood that in performing the present disclosure, separation of cells carrying any given cell surface marker can be effected by a number of different methods, however, some methods rely upon binding a binding agent (e.g., an antibody or antigen binding fragment thereof) to the marker concerned followed by a separation of those that exhibit binding, being either high level binding, or low level binding or no binding. The most convenient binding agents are antibodies or antibody-based molecules, such as monoclonal antibodies or based on monoclonal antibodies because

of the specificity of these latter agents. Antibodies can be used for both steps, however other agents might also be used, thus ligands for these markers may also be employed to enrich for cells carrying them, or lacking them.

The antibodies or ligands may be attached to a solid support to allow for a crude separation. The separation techniques preferably maximize the retention of viability of the fraction to be collected. Various techniques of different efficacy may be employed to obtain relatively crude separations. The particular technique employed will depend upon efficiency of separation, associated cytotoxicity, ease and speed of performance, and necessity for sophisticated equipment and/or technical skill. Procedures for separation may include, but are not limited to, magnetic separation, using antibody-coated magnetic beads, affinity chromatography and "panning" with antibody attached to a solid matrix. Techniques providing accurate separation include but are not limited to FACS. Methods for performing FACS will be apparent to the skilled artisan.

Antibodies against each of the markers described herein are commercially available (e.g., monoclonal antibodies against STRO-1 are commercially available from R&D Systems, USA), available from ATCC or other depositary organization and/or can be produced using art recognized techniques.

The method for isolating STRO-1$^+$ cells, for example, comprises a first step being a solid phase sorting step utilizing for example magnetic activated cell sorting (MACS) recognizing high level expression of STRO-1. A second sorting step can then follow, should that be desired, to result in a higher level of precursor cell expression as described in patent specification WO 01/14268. This second sorting step might involve the use of two or more markers.

The method obtaining STRO-1$^+$ cells might also include the harvesting of a source of the cells before the first enrichment step using known techniques. Thus the tissue will be surgically removed. Cells comprising the source tissue will then be separated into a so called single cells suspension. This separation may be achieved by physical and or enzymatic means.

Once a suitable STRO-1$^+$ cell population has been obtained, it may be cultured or expanded by any suitable means to obtain MEMPs.

In one example, the cells are taken from the subject to be treated, cultured *in vitro* using standard techniques and used to obtain supernatant or soluble factors or expanded cells for administration to the subject as an autologous or allogeneic composition. In an alternative example, cells of one or more of the established human cell lines are used to obtain the supernatant or soluble factors. In another useful example of the disclosure, cells of a non-human animal (or if the patient is not a human, from another species) are used to obtain supernatant or soluble factors.

The disclosure can be practiced using cells from any non-human animal species, including but not limited to non-human primate cells, ungulate, canine, feline, lagomorph, rodent, avian, and fish cells. Primate cells with which the disclosure may be performed include but are not limited to cells of chimpanzees, baboons, cynomolgus monkeys, and any other New or Old World monkeys. Ungulate cells with which the disclosure may be performed include but are not limited to cells of bovines, porcines, ovines, caprines, equines, buffalo and bison. Rodent cells with which the disclosure may be performed include but are not limited to mouse, rat, guinea pig, hamster and gerbil cells. Examples of lagomorph species with which the disclosure may be performed include domesticated rabbits, jack rabbits, hares, cottontails, snowshoe rabbits, and pikas. Chickens (*Gallus gallus*) are an example of an avian species with which the disclosure may be performed.

Cells useful for the methods of the disclosure may be stored before use, or before obtaining the supernatant or soluble factors. Methods and protocols for preserving and storing of eukaryotic cells, and in particular mammalian cells, are known in the art (cf., for example, Pollard, J. W. and Walker, J. M. (1997) Basic Cell Culture Protocols, Second Edition, Humana Press, Totowa, N.J.; Freshney, R. I. (2000) Culture of Animal Cells, Fourth Edition, Wiley-Liss, Hoboken, N.J.). Any method maintaining the biological activity of the isolated stem cells such as mesenchymal stem/progenitor cells, or progeny thereof, may be utilized in connection with the present disclosure. In one example, the cells are maintained and stored by using cryo-preservation.

Genetically-modified cells

In one embodiment, the STRO-1^+ cells and/or progeny cells thereof are genetically modified, e.g., to express and/or secrete a protein of interest, e.g., a protein providing a therapeutic and/or prophylactic benefit, e.g., insulin, glucagon, somatostatin,

trypsinogen, chymotrypsinogen, elastase, carboxypeptidase, pancreatic lipase or amylase or a polypeptide associated with or causative of enhanced angiogenesis or a polypeptide associated with differentiation of a cell into a pancreatic cell or a vascular cell.

Methods for genetically modifying a cell will be apparent to the skilled artisan. For example, a nucleic acid that is to be expressed in a cell is operably-linked to a promoter for inducing expression in the cell. For example, the nucleic acid is linked to a promoter operable in a variety of cells of a subject, such as, for example, a viral promoter, e.g., a CMV promoter (e.g., a CMV-IE promoter) or a SV-40 promoter. Additional suitable promoters are known in the art and shall be taken to apply *mutatis mutandis* to the present embodiment of the invention.

Preferably, the nucleic acid is provided in the form of an expression construct. As used herein, the term "expression construct" refers to a nucleic acid that has the ability to confer expression on a nucleic acid (e.g. a reporter gene and/or a counter-selectable reporter gene) to which it is operably connected, in a cell. Within the context of the present invention, it is to be understood that an expression construct may comprise or be a plasmid, bacteriophage, phagemid, cosmid, virus sub-genomic or genomic fragment, or other nucleic acid capable of maintaining and/or replicating heterologous DNA in an expressible format.

Methods for the construction of a suitable expression construct for performance of the invention will be apparent to the skilled artisan and are described, for example, in Ausubel et al (In: Current Protocols in Molecular Biology. Wiley Interscience, ISBN 047 150338, 1987) or Sambrook et al (In: Molecular Cloning: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Third Edition 2001). For example, each of the components of the expression construct is amplified from a suitable template nucleic acid using, for example, PCR and subsequently cloned into a suitable expression construct, such as for example, a plasmid or a phagemid.

Vectors suitable for such an expression construct are known in the art and/or described herein. For example, an expression vector suitable for the method of the present invention in a mammalian cell is, for example, a vector of the pcDNA vector suite supplied by Invitrogen, a vector of the pCI vector suite (Promega), a vector of the

pCMV vector suite (Clontech), a pM vector (Clontech), a pSI vector (Promega), a VP 16 vector (Clontech) or a vector of the pcDNA vector suite (Invitrogen).

The skilled artisan will be aware of additional vectors and sources of such vectors, such as, for example, Invitrogen Corporation, Clontech or Promega.

Means for introducing the isolated nucleic acid molecule or a gene construct comprising same into a cell for expression are known to those skilled in the art. The technique used for a given organism depends on the known successful techniques. Means for introducing recombinant DNA into cells include microinjection, transfection mediated by DEAE-dextran, transfection mediated by liposomes such as by using lipofectamine (Gibco, MD, USA) and/or cellfectin (Gibco, MD, USA), PEG-mediated DNA uptake, electroporation and microparticle bombardment such as by using DNA-coated tungsten or gold particles (Agracetus Inc., WI, USA) amongst others.

Alternatively, an expression construct of the invention is a viral vector. Suitable viral vectors are known in the art and commercially available. Conventional viral-based systems for the delivery of a nucleic acid and integration of that nucleic acid into a host cell genome include, for example, a retroviral vector, a lentiviral vector or an adeno-associated viral vector. Alternatively, an adenoviral vector is useful for introducing a nucleic acid that remains episomal into a host cell. Viral vectors are an efficient and versatile method of gene transfer in target cells and tissues. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues.

For example, a retroviral vector generally comprises cis-acting long terminal repeats (LTRs) with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of a vector, which is then used to integrate the expression construct into the target cell to provide long term expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), simian immunodeficiency virus (SrV), human immunodeficiency virus (HIV), and combinations thereof (see, e.g., Buchscher *et al., J Virol. 56*:2731-2739 (1992); Johann *et al, J. Virol. 65*:1635-1640 (1992); Sommerfelt *et al, Virol. 76*:58-59 (1990); Wilson *et al, J. Virol. 63*:274-2318 (1989); Miller *et al., J. Virol. 65*:2220-2224 (1991); PCT/US94/05700; Miller and Rosman *BioTechniques 7*:980-990, 1989; Miller, A. D. *Human Gene Therapy 7*:5-14,

1990; Scarpa *et al Virology 75*:849-852, 1991; Burns *et al. Proc. Natl. Acad. Sci USA 90*:8033-8037, 1993).

Various adeno-associated virus (AAV) vector systems have also been developed for nucleic acid delivery. AAV vectors can be readily constructed using techniques known in the art. See, e.g., U.S. Pat. Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 and WO 93/03769; Lebkowski *et al. Molec. Cell. Biol. 5*:3988-3996, 1988; Vincent et al. (1990) Vaccines 90 (Cold Spring Harbor Laboratory Press);Carter *Current Opinion in Biotechnology 5*:533-539, 1992; Muzyczka. *Current Topics in Microbiol, and Immunol. 158*:97-129, 1992; Kotin, Human Gene Therapy 5:793-801, 1994; Shelling and Smith *Gene Therapy 7*:165-169, 1994; and Zhou *et al. J Exp. Med. 179*:1867-1875, 1994.

Additional viral vectors useful for delivering an expression construct of the invention include, for example, those derived from the pox family of viruses, such as vaccinia virus and avian poxvirus or an alphavirus or a conjugate virus vector (e.g. that described in Fisher-Hoch *et al., Proc. Natl Acad. Sci. USA 56*:317-321, 1989).

Assaying Therapeutic/Prophylactic Potential of Cells and Soluble Factors

Methods for determining the ability of soluble factors derived from STRO-1^{bright} cells to suppress T-cell activation will be apparent to the skilled artisan.

For example, suitable *in vitro* tests for determining immunosuppressive activity of the soluble factors are described in Examples 5 and 8 herein.

In another example, efficacy of cells and/or soluble factors described herein is assessed in an *in vivo* model of experimental inflammatory encephalomyelitis (EAE) as described in Example 6 herein.

It will be apparent to the skilled artisan from the foregoing that the present disclosure also provides a method for identifying or isolating a soluble factor for suppressing T cell activation, the method comprising:

(i) administering a a soluble factor to a test subject suffering from EAE and assessing progression of EAE in the subject;

(ii) comparing level of EAE in the subject at (i) to the level EAE in a control subject suffering from EAE to which the soluble factor has not been administered,
wherein reduced EAE in the test subject compared to the control subject indicates that the soluble factor treats, prevents or delays EAE.

Cellular compositions

In one embodiment of the present invention STRO-1^+ cells and/or progeny cells thereof are administered in the form of a composition. Preferably, such a composition comprises a pharmaceutically acceptable carrier and/or excipient.

The terms "carrier" and "excipient" refer to compositions of matter that are conventionally used in the art to facilitate the storage, administration, and/or the biological activity of an active compound (see, e.g., Remington's Pharmaceutical Sciences, 16th Ed., Mac Publishing Company (1980). A carrier may also reduce any undesirable side effects of the active compound. A suitable carrier is, for example, stable, e.g., incapable of reacting with other ingredients in the carrier. In one example, the carrier does not produce significant local or systemic adverse effect in recipients at the dosages and concentrations employed for treatment.

Suitable carriers for this invention include those conventionally used, e.g., water, saline, aqueous dextrose, lactose, Ringer's solution, a buffered solution, hyaluronan and glycols are preferred liquid carriers, particularly (when isotonic) for solutions. Suitable pharmaceutical carriers and excipients include starch, cellulose, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, glycerol, propylene glycol, water, ethanol, and the like.

In another example, a carrier is a media composition, e.g., in which a cell is grown or suspended. Preferably, such a media composition does not induce any adverse effects in a subject to whom it is administered.

Preferred carriers and excipients do not adversely affect the viability of a cell and/or the ability of a cell to reduce, prevent or delay pancreatic dysfunction.

In one example, the carrier or excipient provides a buffering activity to maintain the cells and/or soluble factors at a suitable pH to thereby exert a biological activity, e.g., the carrier or excipient is phosphate buffered saline (PBS). PBS represents an attractive carrier or excipient because it interacts with cells and factors minimally and permits rapid release of the cells and factors, in such a case, the composition of the invention may be produced as a liquid for direct application to the blood stream or into a tissue or a region surrounding or adjacent to a tissue, e.g., by injection.

STRO-1$^+$ cells and/or progeny cells thereof can also be incorporated or embedded within scaffolds that are recipient-compatible and which degrade into products that are not harmful to the recipient. These scaffolds provide support and protection for cells that are to be transplanted into the recipient subjects. Natural and/or synthetic biodegradable scaffolds are examples of such scaffolds.

A variety of different scaffolds may be used successfully in the practice of the invention. Preferred scaffolds include, but are not limited to biological, degradable scaffolds. Natural biodegradable scaffolds include collagen, fibronectin, and laminin scaffolds. Suitable synthetic material for a cell transplantation scaffold should be able to support extensive cell growth and cell function. Such scaffolds may also be resorbable. Suitable scaffolds include polyglycolic acid scaffolds, e.g., as described by Vacanti, *et al. J. Ped. Surg. 23*:3-9 1988; Cima, *et al. Biotechnol. Bioeng. 38*:145 1991; Vacanti, *et al. Plast. Reconstr. Surg. 88*:753-9 1991; or synthetic polymers such as polyanhydrides, polyorthoesters, and polylactic acid.

In another example, the cells may be administered in a gel scaffold (such as Gelfoam from Upjohn Company).

The cellular compositions useful for the present invention may be administered alone or as admixtures with other cells. Cells that may be administered in conjunction with the compositions of the present invention include, but are not limited to, other multipotent or pluripotent cells or stem cells, or bone marrow cells. The cells of different types may be admixed with a composition of the invention immediately or shortly prior to administration, or they may be co-cultured together for a period of time prior to administration.

Preferably, the composition comprises an effective amount or a therapeutically or prophylactically effective amount of cells. For example, the composition comprises about $1x10^5$ STRO-1^+ cells/kg to about $1x10^7$ STRO-1^+ cells/kg or about $1x10^6$ STRO-1^+ cells/kg to about $5x10^6$ STRO-1^+ cells/kg. The exact amount of cells to be administered is dependent upon a variety of factors, including the age, weight, and sex of the patient, and the extent and severity of the pancreatic dysfunction.

In some embodiments, cells are contained within a chamber that does not permit the cells to exit into a subject's circulation, however that permits factors secreted by the cells to enter the circulation. In this manner soluble factors may be administered to a subject by permitting the cells to secrete the factors into the subject's circulation. Such a chamber may equally be implanted at a site in a subject to increase local levels of the soluble factors, e.g., implanted in or near a transplanted organ.

In some embodiments of the invention, it may not be necessary or desirable to immunosuppress a patient prior to initiation of therapy with cellular compositions. Accordingly, transplantation with allogeneic, or even xenogeneic, Stro-1^{bri} cells or progeny thereof may be tolerated in some instances.

However, in other instances it may be desirable or appropriate to pharmacologically immunosuppress a patient prior to initiating cell therapy. This may be accomplished through the use of systemic or local immunosuppressive agents, or it may be accomplished by delivering the cells in an encapsulated device. The cells may be encapsulated in a capsule that is permeable to nutrients and oxygen required by the cell and therapeutic factors the cell is yet impermeable to immune humoral factors and cells. Preferably the encapsulant is hypoallergenic, is easily and stably situated in a target tissue, and provides added protection to the implanted structure. These and other means for reducing or eliminating an immune response to the transplanted cells are known in the art. As an alternative, the cells may be genetically modified to reduce their immunogenicity.

Compositions of soluble factors

In one embodiment of the present invention, STRO-1^+ cell-derived and/or progeny cell-derived supernatant or soluble factors are administered in the form of a composition,

e.g., comprising a suitable carrier and/or excipient. Preferably, the carrier or excipient does not adversely affect the biological effect of the soluble factors or supernatant.

In one embodiment, the composition comprises a composition of matter to stabilize a soluble factor or a component of supernatant, e.g., a protease inhibitor. Preferably, the protease inhibitor is not included in an amount sufficient to have an adverse effect on a subject.

Compositions comprising STRO-1$^+$ cell-derived and/or progeny cell-derived supernatant or soluble factors may be prepared as appropriate liquid suspensions, e.g., in culture medium or in a stable carrier or a buffer solution, e.g., phosphate buffered saline. Suitable carriers are described herein above. In another example, suspensions comprising STRO-1$^+$ cell-derived and/or progeny cell-derived supernatant or soluble factors are oily suspensions for injection. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil; or synthetic fatty acid esters, such as ethyl oleate or triglycerides; or liposomes. Suspensions to be used for injection may also contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Sterile injectable solutions can be prepared by incorporating the supernatant or soluble factors in the required amount in an appropriate solvent with one or a combination of ingredients described above, as required, followed by filtered sterilization.

Generally, dispersions are prepared by incorporating the supernatant or soluble factors into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. In accordance with an alternative aspect of the invention, the supernatant or soluble factors may be formulated with one or more additional compounds that enhance its solubility.

Other exemplary carriers or excipients are described, for example, in Hardman, et al. (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N. Y.; Gennaro (2000) Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N. Y.; Avis, et al. (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY; Weiner and Kotkoskie (2000) Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N. Y.

Therapeutic compositions typically should be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin. Moreover, the soluble factors may be administered in a time release formulation, for example in a composition which includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are patented or generally known to those skilled in the art.

The supernatant or soluble factors may be administered in combination with an appropriate matrix, for instance, to provide slow release of the soluble factors.

Modes of administration

The STRO-1$^+$cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof may be surgically implanted, injected, delivered (e.g., by way of a catheter or syringe), or otherwise administered directly or indirectly to the site in need of repair or augmentation, e.g., an organ or into the blood system of a subject.

Preferably, the STRO-1$^+$ cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof is delivered to the blood stream of a subject. For example, the Stro-1^{bri} cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof are delivered parenterally. Exemplary routes of parenteral administration include, but are not limited to, intravenous, intramuscular, subcutaneous, intra-arterial, intraperitoneal, intraventricular, intracerebroventricular, intrathecal. Preferably, the STRO-1$^+$ cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof are delivered intra-arterially, into an aorta, into an atrium or ventricle of the heart or into a blood vessel connected to a pancreas, e.g., an abdominal aorta, a superior mesenteric artery, a pancreaticoduodenal artery or a splenic artery.

In the case of cell delivery to an atrium or ventricle of the heart, it is preferred that cells are administered to the left atrium or ventricle to avoid complications that may arise from rapid delivery of cells to the lungs.

Preferably, the STRO-1$^+$ cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof are injected into the site of delivery, e.g., using a syringe or through a catheter or a central line.

Selecting an administration regimen for a therapeutic formulation depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, and the immunogenicity of the entity. Preferably, an administration regimen maximizes the amount of therapeutic compound delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of formulation delivered depends in part on the particular entity and the severity of the condition being treated.

In one embodiment, STRO-1$^+$ cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof are delivered as a single bolus dose. Alternatively, STRO-1$^+$ cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof are

administered by continuous infusion, or by doses at intervals of, e.g., one day, one week, or 1-7 times per week. A preferred dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects. A total weekly dose depends on the type and activity of the compound being used. Determination of the appropriate dose is made by a clinician, e.g., using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of diabetes.

EXAMPLES

Example 1: MSC preparation

MSCs are generated de novo from bone marrow as described in US 5,837,539. Approximately 80-100 ml of marrow was aspirated into sterile heparin-containing syringes and taken to the MDACC Cell Therapy Laboratory for MSC generation. The bone marrow mononuclear cells were isolated using ficoll-hypaque and placed into twoT175 flask with 50 ml per flask of MSC expansion medium which includes alpha modified MEM (αMEM) containing gentamycin, glutamine (2 mM) and 20% (v/v) fetal bovine serum (FBS) (Hyclone).

The cells were cultured for 2-3 days in 37°C, 5%CO_2 at which time the non-adherent cells were removed; the remaining adherent cells were continually cultured until the cell confluence reached 70% or higher (7-10 days), and then the cells were trypsinized and replaced in six T175 flasks with MSC expansion medium (50 ml of medium per flask). As described in Table 5 of US 5,837,539, MSCs isolated and expanded in this manner are STRO-1 negative.

Example 2: Immunoselection of MPCs by selection of STRO-3+ cells

Bone marrow (BM) is harvested from healthy normal adult volunteers (20-35 years old), in accordance with procedures approved by the Institutional Ethics Committee of the Royal Adelaide Hospital. Briefly, 40 ml of BM is aspirated from the posterior iliac crest into lithium-heparin anticoagulant-containing tubes.

BMMNC are prepared by density gradient separation using Lymphoprep™ (Nycomed Pharma, Oslo, Norway) as previously described (Zannettino, A.C. *et al.* (1998) *Blood* 92: 2613-2628). Following centrifugation at 400 x g for 30 minutes at 4°C, the buffy layer is removed with a transfer pipette and washed three times in "HHF", composed of Hank's balanced salt solution (HBSS; Life Technologies, Gaithersburg, MD), containing 5% fetal calf serum (FCS, CSL Limited, Victoria, Australia).

STRO-3$^+$ (or TNAP$^+$) cells were subsequently isolated by magnetic activated cell sorting as previously described (Gronthos *et al.* (2003) *Journal of Cell Science* 116: 1827-1835; Gronthos, S. and Simmons, P.J. (1995) *Blood* 85: 929-940). Briefly, approximately 1-3 x 10^8 BMMNC are incubated in blocking buffer, consisting of 10% (v/v) normal rabbit serum in HHF for 20 minutes on ice. The cells are incubated with 200µl of a 10µg/ml solution of STRO-3 mAb in blocking buffer for 1 hour on ice. The cells are subsequently washed twice in HHF by centrifugation at 400 x g. A 1/50 dilution of goat anti-mouse γ-biotin (Southern Biotechnology Associates, Birmingham, UK) in HHF buffer is added and the cells incubated for 1 hour on ice. Cells are washed twice in MACS buffer (Ca^{2+} - and Mn^{2+} -free PBS supplemented with 1% BSA, 5 mM EDTA and 0.01% sodium azide) as above and resuspended in a final volume of 0.9 ml MACS buffer.

One hundred µl streptavidin microbeads (Miltenyi Biotec; Bergisch Gladbach, Germany) are added to the cell suspension and incubated on ice for 15 minutes. The cell suspension is washed twice and resuspended in 0.5 ml of MACS buffer and subsequently loaded onto a mini MACS column (MS Columns, Miltenyi Biotec), and washed three times with 0.5 ml MACS buffer to retrieve the cells which did not bind the STRO-3 mAb (deposited on 19 December 2005 with American Type Culture Collection (ATCC) under accession number PTA-7282 - see International Publication No.WO 2006/108229). After addition of a further 1 ml MACS buffer, the column is removed from the magnet and the TNAP$^+$ cells are isolated by positive pressure. An aliquot of cells from each fraction can be stained with streptavidin-FITC and the purity assessed by flow cytometry.

Example 3: Cells selected by STRO-3 mAb are STRO-1^{bright} cells

Experiments were designed to confirm the potential of using STRO-3 mAb as a single reagent for isolating cells STRO-1^{bright} cells.

Given that STRO-3 (IgG1) is a different isotype to that of STRO-1 (IgM), the ability of STRO-3 to identify clonogenic CFU-F was assessed by two-colour FACS analysis based on its co-expression with STRO-1$^+$ cells isolated using the MACS procedure (Figure 1). The dot plot histogram represents 5 x 10^4 events collected as listmode data. The vertical and horizontal lines were set to the reactivity levels of <1.0% mean fluorescence obtained with the isotype-matched control antibodies, 1B5 (IgG) and 1A6.12 (IgM) treated under the same conditions. The results demonstrate that a minor population of STRO-1bright cells co-expressed TNAP (upper right quadrant) while the remaining STRO-1$^+$ cells failed to react with the STRO-3 mAb. Cells isolated by FACS from all four quadrants were subsequently assayed for the incidence of CFU-F (Table 1).

Table 1: Enrichment of human bone marrow cells by dual-colour FACS analysis based on the co-expression of the cell surface markers STRO-1 and TNAP (refer to Figure 1). FACS sorted cells were cultured under standard clonogenic conditions in alpha MEM supplemented with 20% FCS. The data represents the mean number of day 14 colony-forming cells (CFU-F) per 10^5 cells plated ± SE (n=3 different bone marrow aspirates). These data suggest that human MPC are exclusively restricted to the TNAP positive fraction of BM which co-express the STRO-1 antigen brightly.

Bone Marrow Fraction	Frequency of CFU-F/10^5 Cells	Enrichment (Fold Increase)
Unfractionated BMMNC	11.0 ± 2.2	1.0
TNAP+/STRO-1bright	4,511 ± 185	410
TNAP+/STRO-1dull	0.0	0.0

Example 4: Relative gene and surface protein expression of Stro-1^{dull} and Stro-1^{bright} cells

In the first series of experiments, semi-quantitative RT-PCR analysis was employed to examine the gene expression profile of various lineage-associated genes expressed by STRO-1^{dull} or STRO-1^{bright} populations, isolated by fluorescence activated cell sorting (Figure 2A). In the second series of experiments, flow cytometry and mean channel fluorescence analysis was employed to examine the surface protein xpression profile of various lineage-associated proteins expressed by STRO-1^{dull} or STRO-1^{bright} populations, isolated by fluorescence activated cell sorting.

Total cellular RNA was prepared from either 2 x 10^6 STRO-1^{bright} or STRO-1^{dull} sorted primary cells, chondrocyte pellets and other induced cultures and lysed using RNAzolB extraction method (Biotecx Lab. Inc., Houston, TX), according to the manufacturer's recommendations. RNA isolated from each subpopulation was then used as a template for cDNA synthesis, prepared using a First-strand cDNA synthesis kit (Pharmacia Biotech, Uppsala, Sweden). The expression of various transcripts was assessed by PCR amplification, using a standard protocol as described previously (Gronthos *et al.*, J. Bone and Min. Res. 14:48-57, 1999). Primer sets used in this study are shown in Table 2. Following amplification, each reaction mixture was analysed by 1.5% agarose gel electrophoresis, and visualised by ethidium bromide staining. RNA integrity was assessed by the expression of GAPDH.

Relative gene expression for each cell marker was assessed with reference to the expression of the house-keeping gene, GAPDH, using ImageQant software (Figure 2B, C). In addition, dual-colour flow cytometric analysis was used to examine the protein expression profile of *ex vivo* expanded MPC based on their expression of a wider range of cell lineage-associated markers in combination with the STRO-1 antibody. A summary of the general phenotype based on the gene and protein expression of STRO-1^{dull} and STRO-1^{bright} cultured cells is presented in Table 3. The data indicate that *ex vivo* expanded STRO-1^{bright} MPC exhibit differentially higher expression of markers associated with perivascular cells, including angiopoietin-1, VCAM-1, SDF-1, IL-1β, TNFα, and RANKL. Comparisons between the protein and gene expression profiles of STRO-1^{dull} and STRO-1^{bri} cultured cells are summarised in Tables 3 and 4.

Subtractive hybridization studies were also performed in order to identify genes uniquely expressed by STRO-1^{bright} cells. Briefly, STRO-1^{dull} and STRO-1^{bright} were isolated as described above (see Figure 3A). Total RNA was prepared from STRO-1^{dull} and STRO-1^{bright} cells pooled from 5 different marrow samples using the RNA STAT-60 system (TEL-TEST). First-strand synthesize was performed using the SMART cDNA synthesis kit (Clontech Laboratories). The resultant mRNA/single-stranded cDNA hybrid was amplified by long-distance PCR (Advantage 2 PCR kit; Clontech) using specific primer sites at the 3' and 5' prime ends formed during the initial RT process according to the manufacturer's specifications. Following RsaI digestion of the STRO-1bright cDNA, 2 aliquots were used to ligate different specific adaptor oligonucleotides using the Clontech PCR-Select cDNA Subtraction Kit. Two rounds of subtractive hybridization were performed using STRO-1^{bright} (tester) and STRO-1^{dull}

(driver) cDNA, and vice versa, according to the manufacturer's protocol. This procedure was also performed in reverse using STRO-1^{dull} tester cDNA hybridized against STRO-1^{bright} driver cDNA.

To identify genes uniquely expressed by STRO-1^{bright} population, STRO-1^{bright}–subtracted cDNA was used to construct replicate low-density microarray filters comprising 200 randomly selected bacterial clones transformed with the STRO-1^{bri}subtracted cDNAs ligated into a T/A cloning vector. The microarrays were subsequently probed with either [^{32}P] dCTP–labeled STRO-1^{bri} or STRO-1^{dull} subtracted cDNA (Figure 3B-C). Differential screening identified a total of 44 clones, which were highly differentially expressed between the STRO-1^{dull} and STRO-1^{bright} subpopulations. DNA sequencing of all the differentially expressed clones revealed that only 1 clone was representative of a known stromal cell mitogen; namely, platelet-derived growth factor (PDGF) (Gronthos and Simmons, Blood. 85: 929-940, 1995). Interestingly, 6 of the 44 clones were found to contain DNA inserts corresponding to the chemokine, stromal-derived factor-1 (SDF-1). The high abundance of SDF-1 transcripts in human STRO-1^{bright} cells was confirmed by semiquantitative RT-PCR of total RNAprepared from freshly sorted STRO-1^{bright}, STRO-1^{dull}, and STRO-1^{negative} bone marrow subpopulations (Figure 3D and Table 3).

Table 2. RT-PCR primers and conditions for the specific amplification of human mRNA

Target Gene	Sense/ Antisense (5'-3') Primer Sequences	Product Size
GAPDH	CACTGACACGTTGGCAGTGG/ CATGGAGAAGGCTGGGGCTC	417
SDF-1	GAGACCCGCGCTCGTCCGCC/ GCTGGACTCCTACTGTAAGGG	364
IL-1β	AGGAAGATGCTGGTTCCCTCTC/ CAGTTCAGTGATCGTACAGGTGC	151
FLT-1	TCACTATGGAAGATCTGATTTCTTACAGT/ GGTATAAATACACATGTGCTTCTAG	380

Target Gene	Sense/ Antisense (5'-3') Primer Sequences	Product Size
TNF-α	TCAGATCATCTTCTCGAACC/ CAGATAGATGGGCTCATACC	361
KDR	TATAGATGGTGTAACCCGGA/ TTTGTCACTGAGACAGCTTGG	450
RANKL	AACAGGCCTTTCAAGGAGCTG/ TAAGGAGGGGTTGGAGACCTCG	538
Leptin	ATGCATTGGGAACCCTGTGC/ GCACCCAGGGCTGAGGTCCA	492
CBFA-1	GTGGACGAGGCAAGAGTTTCA/ TGGCAGGTAGGTGTGGTAGTG	632
PPARγ2	AACTGCGGGGAAACTTGGGAGATTCTCC/ AATAATAAGGTGGAGATGCAGGCTCC	341
OCN	ATGAGAGCCCTCACACTCCTC/ CGTAGAAGCGCCGATAGGC	289
MyoD	AAGCGCCATCTCTTGAGGTA/ GCGAGAAACGTGAACCTAGC	270
SMMHC	CTGGGCAACGTAGTAAAACC/ TATAGCTCATTGCAGCCTCG	150
GFAP	CTGTTGCCAGAGATGGAGGTT/ TCATCGCTCAGGAGGTCCTT	370
Nestin	GGCAGCGTTGGAACAGAGGTTGGA/ CTCTAAACTGGAGTGGTCAGGGCT	460
SOX9	CTCTGCCTGTTTGGACTTTGT/ CCTTTGCTTGCCTTTTACCTC	598
Collagen type X	AGCCAGGGTTGCCAGGACCA/ TTTTCCCACTCCAGGAGGGC	387
Aggrecan	CACTGTTACCGCCACTTCCC/ ACCAGCGGAAGTCCCCTTCG	184

Table 3. Summary of the Relative Gene Expression in STRO-1^{Bri} and STRO-1^{Dull} populations. A list of genes which displayed measurable and differential expression between the STRO-1^{Bri} and STRO-1^{Dull} populations as determined by reverse transcription-PCR are presented . Values represent the relative gene expression with reference to the house-keeping gene, GAPDH.

		Gene Expression relative to GAPDH	
Tissue	**Marker**	**STRO-1^{Bri}**	**STRO-1^{Dull}**
Neurons	*GFAP (Glial Fibrillary Acidic Protein)*	0.1	0.7
Bone	*OCN (Osteocalcin)*	1.1	2.5
	OSX (Osterix)	0.4	1.3
	CBFA-1 (Core Factor Binding Protein-1)	0.3	0.6
Immunoregulatory	*RANKL (Receptor Activator of Nuclear Factor κ B)*	1.6	0.3
	SDF-1-alpha (Stromal Derived factor-1-alpha)	3.2	0.1
Fat	*Leptin*	3.1	4.2
Cardiomyocytes	*GATA-4*	1.1	2.9
Endothelial cells	*Ang-1 (Angiopoietin-1)*	1.5	0.8
Chondrocytes	*Sox 9*	0.3	1.1
	COL X (Collagen X)	3.5	2.8
Pro-inflammatory Cytokines	*TNF-alpha (Tumour necrosis alpha)*	1.7	0.9

To correlate protein surface expression with density of STRO-1 expression, single cell suspensions of *ex vivo* expanded cells derived bone marrow MPC were prepared by trypsin/EDTA detachment and subsequently incubated with the STRO-1 antibody in combination with antibodies identifying a wide range of cell lineage-associated markers. STRO-1 was identified using a goat anti-murine IgM-fluorescein isothiocyanate while all other markers were identified using either a goat anti-mouse or anti-rabbit IgG- phycoerythrin. For those antibodies identifying intracellular antigens, cell preparations were first labelled with the STRO-1 antibody, fixed with cold 70% ethanol to permeabilize the cellular membrane and then incubated with intracellular antigen-specific antibodies. Isotype matched control antibodies were used under identical conditions. Dual-colour flow cytometric analysis was performed using a COULTER EPICS flow cytometer and list mode data collected. The dot plots represent 5,000 listmode events indicating the level of fluorescence intensity for each lineage cell marker (y-axis) and STRO-1 (x-axis). The vertical and horizontal quadrants were established with reference to the isotype matched negative control antibodies.

Table 4. Summary of the Relative Protein Expression in STRO-1^{Bright} and STRO-1^{Dull} populations. A list of proteins which displayed differential expression between the STRO-1^{Bright} and STRO-1^{Dull} populations as determined by flow cytometry are presented. Values represent the relative mean fluorescence intensity of staining.

		Mean Fluorescence Intensity	
Tissue	**Marker**	**STRO-1^{Bright}**	**STRO-1^{Dull}**
Neurons	*Neurofilament*	1.7	20.5
Bone	*ALK PHOS (Alkaline Phophatase)*	5.7	44.5
Immunoregulatory	*RANKL (Receptor Activator of Nuclear Factor κ B)*	658.5	31.0
Epithelial Cells	*CytoKeratin 10+13*	1.2	23.3
	Cytokeratin 14	1.8	8.8
Smooth Muscle	*α-SMA (Alpha Smooth Muscle Actin)*	318.0	286.0
Chondrocytes	*Byglycan*	84.4	65.9
Basal Fibroblast	*Tenascin C*	22.2	6.9
Cardiomyocyte	*Troponin C*	2.5	15.0

These results show that SDF-1alpha and RANKL are highly expressed by STRO-1^{bright} cells. This is important because both of these proteins are known to be involved in up-regulation of CD4+ CD25+ regulatory T cells which confer protection against immune disorders such as GVHD (Loser *et al.*, Nature Medicine 12:1372-1379, 2006; Hess, Biol. Blood Marrow Transplant, 12 (1 Suppl 2):13-21, 2006; and Meiron *et al.*, J. Exp. Medicine 205:2643-2655, 2008).

Example 5. *In vitro* immunosuppressive activity

To assess immunosuppressive activity of culture-expanded STRO-1^{bright} cells (MPC(B)), we used CD3/CD28 stimulation as a read-out. Results were compared to a population of culture-expanded, bone marrow-derived STRO-1 negative cells isolated as in Example 1 (MSC(A)). Human peripheral blood mononuclear cells (PBMC) were stimulated with CD3/CD28 coated beads in the presence of 4 escalating concentrations of MSC and MPC preparations. The proliferation of T cells was measured by 3H-Tdr incorporation.

MSC (A) and Stro-1^{bright} MPCs (B) were tested for their ability to suppress the response of human peripheral blood mononuclear cells (PBMC) to CD3/CD28 stimulation. MSC and MPC or commercially-purchased control human MSC (Lonza) were added at different ratios to the cultures of PBMC. After 3 days, 3H-Tdr was added for 18 hours and the cultures then harvested.

PBMC proliferation in response to CD3/CD28 was inhibited in a dose dependent fashion by all preparations. However, preparation B was clearly superior to the effect produced by preparation A as well as control hMSC (Figure 4). At a 1:100 MSC:PBMC ratio, MPC B still inhibited 70% of control T cell proliferation, whilst control commercially-purchased MSC (Lonza) and MSC A produced a 50% and 60% inhibition, respectively (Figure 5).

Example 6: *In vivo* effect of MPCs on T cell Proliferation

For the following experiments the myelin oligodendrocyte glycoprotein (MOG)-induced experimental inflammatory encephalomyelitis (EAE) in C57Bl/6J mice was used. C57Bl/6J mice display similar phenotypic symptoms (progressive paralysis) to that of MS patients as well as showing extensive inflammation, demyelination and

axonal loss/damage in the CNS. The immunization procedure for the induction of EAE, assessment of clinical symptoms and MPC transplantation used is as follows.

Active Induction of EAE

Mice were immunized with 200µg recombinant MOG dissolved in Phosphate Buffered Saline (PBS) and mixed with an equal volume of Freund's complete adjuvant containing 400 µg of killed Mycobacterium tuberculosis H37Ra. 0.1 ml of this mixture was injected subcutaneously into the right and left flank (total 0.2 ml/mouse) using a 25 gauge (G) needle. Mice were also immunized with 350ng inactivated *Bordetella* pertussis toxin in 0.30 ml of PBS intravenously (i.v.) via tail vein of on day 0 and day 2 using a 29 G needle. Gentle pressure was applied to the I.V. site for 30 sec after the injection to reduce the risk of bleeding from the i.v. site.

Mice were monitored every 2-5 minutes for 10-15 minutes to ensure there is no active bleeding.

Treatment with MPCs

MPCs were isolated essentially as described in Example 2. On days 8, 10 and 12 after disease induction, $2x10^5$ or $4x10^5$ MPCs were administered as a single intravenous (i.v.) injection in a volume of 200µl PBS (see Table 5). Controls received i.v. injections of equal volumes of PBS only. Mice were monitored daily and clinical signs scored according to the scale described below. Experiments were continued for approximately 36 days to monitor the course of disease. At termination of the experiment, brain, spinal cord and optic nerve were dissected and fixed in formalin solution.

Table 5: Summary of Treatment Regimen

Treatment	**No of cells per mouse per injection**	**Total MPC injected per 20g mouse**	**Number of mice**
PBS I.V.	-	-	12
High dose MPC I.V.	4 x10^5 MPC	6x10^6MPC/Kg	5
Low dose MPC I.V.	2x10^5 MPC	3x10^6MPC/Kg	5

MPC-treated mice and controls were culled on day 36 after disease induction (MOG35-55 immunization). Splenocytes were cultured *in vitro* with media alone or re-stimulated with MOG_{35-55} and then T-cell proliferative responses were measured through [^{3}H]-thymidine incorporation. The specific proliferative responses to MOG were compared to the matched splenocytes cultured in media-alone (unstimulated). Splenocytes cultured in PMA/Ionomycin served to determine the non-specific (antigen-independent) stimulation of T cell proliferation.

Data presented in Figure 6 demonstrate that T cell immune responses to secondary *in vitro* antigenic challenge with MOG are inhibited in comparison to T cells cultured from control animals.

These data show that human MPCs reduce or prevent T cell immune response to a specific antigen (e.g., antigenic stimulation by MOG), even 24 days after the last administration of MPCs. The data indicate that STRO-1 enriched MPC induce tolerance to multiple sclerosis antigens.

Example 7: *In Vitro* Effects of MPCs

The immunoregulatory properties of MPC are tested by proliferation assays, mixed lymphocyte reactions and cytokines production as described below.

Proliferation assays and mixed lymphocyte reactions

Mononuclear cells are collected from the spleens of healthy C57BL/6 mice, 2D2 transgenic mice or MOG-immunized mice treated with MPCs or vehicle alone

essentially as described in Example 4. Single cell suspensions are prepared in complete RPMI media containing 10% FBS, 2mM L-glutamine, 100units/ml penicillin, 100μg/ml streptomycin (all from Invitrogen), 1mM sodium pyruvate (Sigma) and 50μM β-mercaptoethanol (Sigma). Following red blood cell lysis, cells are washed twice and then seeded in 96-well flat bottom microtiter plates (Nunc) in triplicate at a concentration of 2.5 x 10^5 cells per well in the presence of either 20μg/ml MOG_{35-55} (GL Biochem), 800ng/ml ionomycin and 20pg/ml phorbol myristate acetate (PMA) (both from Sigma), or into wells pre-coated with 10μg/ml anti-CD3 and 10μg/ml anti-CD8 (both from BD). Cells are then incubated at 37°C with 5%CO_2 for 72 hours and 1μCi/well [3H] thymidine is added during the last 18 hours of culture. Cells are harvested onto filter mats and incorporated radioactive nucleic acids counted on a Top Count Harvester (Packard Biosciences). For experiments involving inhibition of T-cell proliferation by MPC, concentrations of MPC ranging from 2.5 to 0.002 x 10^4 cells per well are seeded prior to the addition of splenocytes.

In mixed lymphocyte reactions (MLR), 2 x 10^5 splenocytes from C57BL/6 mice (responders) are incubated with equal numbers of irradiated (20Gy) Balb/c stimulators or irradiated MPC and cultured for a period of 5 days, with the addition of 1μCi/well [3H] thymidine during the last 24 hours of culture.

In MLRs involving T-cell inhibition, 2 x 10^4 irradiated MPC are seeded into the wells prior to the addition of splenocytes.

Cytokine production

Supernatants used for analysis of cytokine production are obtained from two day co-cultures of 2.5 x 10^6 splenocytes from 2D2 transgenic mice stimulated with 20μg/ml MOG_{35-55} alone or in the presence of 2 x 10^4 MPC (MPC: splenocyte ratio of 1:10). Quantitative analysis of cytokines us performed using a mouse Th1/Th2/Th17 cytometric bead array (CBA) kit (BD) essentially according to the manufacturer's instructions and analyzed on a BD FACSCanto II flow cytometer. The following cytokines are measured: interleukin (IL)-2, IL-4, IL-6, IL-10, IL-17A, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α).

The data of this pilot study have consistently shown that STRO-1^{bright} MPCs exhibited superior immunosuppressive capacities as compared to either no treatment or treatment

with STRO-1 negative MSCs. This was evident in the *in vitro* assay and, most importantly in the in vivo assay.

Example 8: Effects of MPCs on PHA-mediated lymphocyte proliferation

PBMC were stimulated with phytohemagglutinin (PHA; 10 ug/ml; Sigma Chemical Company, St. Louis, Mo.) to illicit lymphocyte proliferation. STRO-1 bright cells at arrange of concentrations (see Table 6) were able to significantly suppress PBMC T cell proliferative responses as shown in Figure 7.

Table 6: MLR Dilution of Stro-1^{bri} cells

Responder Cell #	Stimulator Cell (Stro-1^{bri}) #	% Stro-1^{bri} cells
50,000/0.1ml	500/0.1ml	1%
50,000/0.1ml	2,500/0.1ml	5%
50,000/0.1ml	5,000/0.1ml	10%
50,000/0.1ml	10,000/0.1ml	20%
50,000/0.1ml	25,000/0.1ml	50%
50,000/0.1ml	50,000/0.1ml	100%

Similar to the human findings ovine MPCs were able to significantly inhibit levels of alloimmune responses to ovine PBMCs stimulated with PHA (Figure 8). Ovine STRO-3 selected cells were also able to suppress lymphocyte proliferation in a dose dependent manner when used as stimulators against ovine PBMCs or purified ovine T cells (selected using Miltenyi T cell isolation kit) (Figure 9).

All references cited in this document are incorporated herein by reference.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Claims

1. A method for suppressing T cell activation which comprises contacting a cell population comprising T cells *in vitro* or *ex vivo* with an effective amount of STRO-1^+ cells and/or soluble factors derived therefrom to suppress T cell activation.

2. A method according to claim 2 wherein the cell population is a peripheral blood mononuclear cell sample.

3. A method according to claim 2 wherein the cell population comprise $CD25^+$ $CD4^+$ T cells of a naïve phenotype ($CD45RA^+$).

4. A method according to any one of claims 1 to 3 which comprises contacting the cell population comprising T cells *in vitro* or *ex vivo* with an effective amount of STRO-1^+ cells and/or soluble factors derived therefrom and one or more factors which induce formation of regulatory T cells.

5. A method according to claim 4 wherein the one or more factors which induce formation of regulatory T cells is selected from the group consisting of α-melanocyte-stimulating hormone (α-MSH), transforming growth factor-β2 (TGF-β2), vitamin D3 and/or Dexamethasone.

6. A method according to any one of claims 1 to 4 which further comprises contacting the cell population comprising T cells with one or more agents selected from the group consisting of interleukins, antigens, antigen presenting cells, lectins, and antibodies or specific ligands for a cell surface receptors or combinations thereof.

7. The method according to claim 6 wherein the interleukin is IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18 or combinations thereof.

8. A composition of T cells obtained by the method of any one of claims 1 to 7.

9. A composition comprising T cells, STRO-1^+ cells and/or soluble factors derived therefrom, one or more factors which induce formation of regulatory T cells and a pharmaceutically acceptable carrier.

10. A method for treating an autoimmune disorder in a subject in need thereof comprising treating a cell population comprising T cells *in vitro* or *ex vivo* with an effective amount of STRO-1^+ cells and/or soluble factors derived therefrom to suppress T cell activation and administering the treated cells to the subject.

11. A method for treating or preventing a disorder caused by excessive or aberrant T cell activation comprising administering to a subject in need thereof an amount of STRO-1^+ cells and/or soluble factors derived therefrom effective to suppress T-cell activation in the patient.

12. The method of claim 10 or claim 11, comprising administering between 0.1 x 10^6 to 5 x 10^6 STRO-1^+ cells and/or progeny thereof.

13. The method of any one of claims 10 to 12, comprising administering between 0.3 x 10^6 to 2 x 10^6 STRO-1^+ cells and/or progeny thereof.

14. The method of any one of claims 10 to 12 comprising administering a low dose of STRO-1^+ cells and/or progeny thereof.

15. The method of claim 14, wherein the low dose of STRO-1^+ cells and/or progeny thereof comprises between 0.1 x 10^5 and 0.5 x 10^6 STRO-1^+ cells and/or progeny thereof..

16. The method of claim 14, wherein the low dose of STRO-1^+ cells and/or progeny thereof comprises about 0.3 x 10^6 STRO-1^+ cells and/or progeny thereof.

17. A method according to any one of claims 10 to 16 which further comprises administering to the subject one or more agents selected from the group consisting of interleukins, antigens, antigen presenting cells, lectins, and antibodies or specific ligands for a cell surface receptors or combinations thereof.

18. The method according to claim 17 wherein the interleukin is IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18 or combinations thereof.

19. The method according to any one of claims 10 to 18, further comprising administering an immunosuppressive drug to the subject.

20. A method according to claim any one of claims 1 to 19 wherein the STRO-1^{+} cells are enriched for STRO-1^{bright} cells.

21. A method according to any one of claims 1 to20 wherein the STRO-1^{bright} cells and/or progeny thereof are allogeneic.